Rocky Mountains   Southwest   Great Plains

Research Note RM-415

June 1982

USDA Forest Service
Rocky Mountain Forest and Range Experiment Station

Pollution Indicator Bacteria in Stream and Potable Water Supply of the Manitou Experimental Forest, Colorado

David C. Erickson, Howard L. Gary, S. M. Morrison, and Glen Sanford[1]

Shallow groundwater flow diverted for domestic use indicated low fecal coliform numbers, and no consistent source of warm-blooded animal fecal material in stream water. Total coliforms and fecal streptococci were observed frequently and increased temporarily in both stream and domestic water supply after summer rains.

Keywords: Water quality, total coliforms, fecal streptococci, fecal coliforms, alluvium

Management Implications

Alluvium in stream bottoms along much of the Front Range in Colorado is of granitic origin and coarse textured. Incomplete filtering of biological pollutants near shallow domestic wells is a good possibility across the area. Results from this study point to a need for periodic bacteriological testing for potential health hazards, upgrading shallow groundwater wells, and disinfecting of drinking water.

Introduction

Samples of drinking water, taken monthly since 1978 at a residence and at other buildings in the Manitou Experimental Forest, have been tested by the Colorado State Health Department. On some occasions, bacterial counts have exceeded allowable limits for potable water supplies (Colorado Water Quality Control Commission 1978), especially during the summer. Possible sources of bacterial pollution were thought to be groundwater contamination from upstream residences, background contamination from endogenous bacterial populations caused by wildlife, and contamination from weekend recreationists. This study was undertaken to investigate the sources of bacterial pollution in the drinking water used at the Manitou Experimental Forest.

Study Area

The Manitou Experimental Forest headquarters is located about 45 km northwest of Colorado Springs, Colo., at an elevation of about 2,385 m above mean sea level. The area is characterized by mild summers, with maximum temperatures seldom over 33° C, and cold, open winters, with minimum temperatures as low as −35° C. Average annual precipitation averages about 400 mm, with two-thirds coming in April through September.

Domestic water for the headquarters comes from Hotel Gulch, a narrow, steep-sloped drainage east of the headquarters (fig. 1). From the point of domestic water diversion (the only diversion on the stream), the up-

[1]Morrison is Professor of Microbiology and Erickson and Sanford (now deceased) were Graduate Research Assistants, Department of Microbiology, Colorado State University, Fort Collins. Gary is Principal Hydrologist, Rocky Mountain Forest and Range Experiment Station, Fort Collins, in cooperation with Colorado State University.

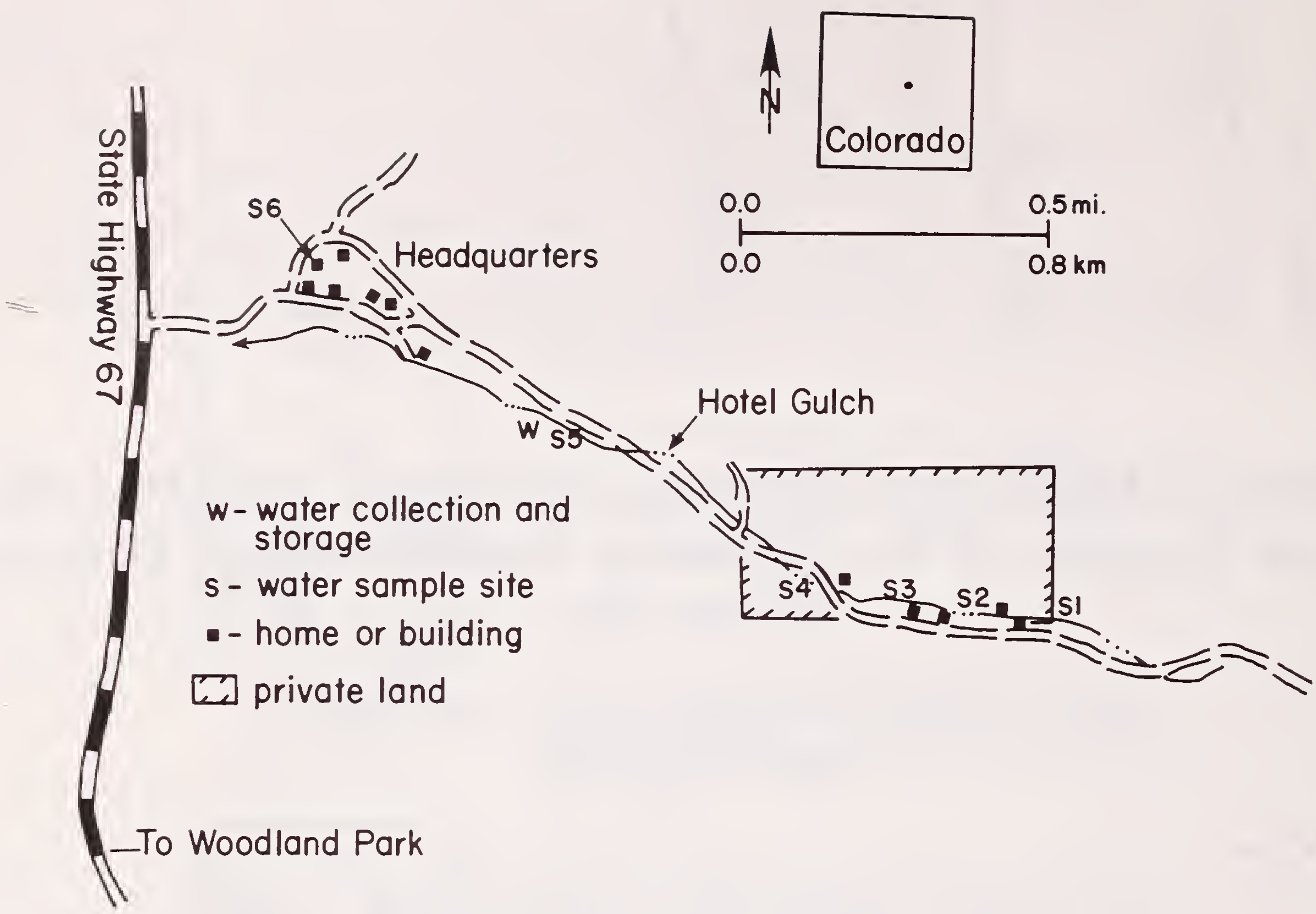

Figure 1.—Location of water sampling sites and physical features of the study area.

stream drainage extends about 5 km eastward to the north-south oriented Rampart Range. Domestic livestock were removed from the watershed about 30 years ago. Vegetation in Hotel Gulch consists mainly of old-growth ponderosa pine on dry, south aspects and old-growth Douglas-fir on the moist, north aspects. The rocky stream bottom is generally well shaded by Engelmann spruce, Douglas-fir, ponderosa pine, and quaking aspen. Understory shrubs, such as thinleaf alder, water birch, common chokecherry, and willows, are common and provide additional shade over the stream.

The stream bottom is mainly coarse-textured alluvium, derived from Pikes Peak granite.[2] Surface streamflow at the point of diversion usually ranges from about 25 l/s at time of peak runoff in May to no surface flow from July through September.

Access into Hotel Gulch is by an unimproved road generally located less than 5 m from the stream for a distance of more than 2 km before it turns away from the stream. Four rustic, recreational or second homes, 0.85-1.55 km above the water diversion, are on private lands along the stream bottom. The homes are all less than 15 m from the stream. Two of the homes have septic tanks and leach fields. The septic tank and leach field for one home are less than 8 m from the stream and less than 1 m above the usual stream level. The other two homes have rundown outdoor toilets. During the period of study, the four homes were occupied only occasionally, but the home with the septic tank nearest the stream bottom had been occupied by several people during most of the previous year. A modern and continually occupied home was located about 200 m below the rustic homes; it has a septic system and leach field about 40 m from the stream channel.

[2]Unpublished manuscript. "Soils and physical conditions of Manitou Experimental Forest," 19 p. plus appendix, by John L. Retzer. USDA Forest Service. On file at Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colo., 1949.

Groundwater Collection and Storage

Groundwater flow in Hotel Gulch is diverted into two, 20-cm, perforated, steel pipes 2-3 m below the ground surface and collected in an underground concrete tank (volume ranges from 3 to 5 m^3) 20 m north of the stream channel. The main collection pipe (46 m long) extends about 13 m beyond the south bank of the stream to the collection tank. The second pipe extends about 22 m northeast and intercepts groundwater in the area. This system has been in use for more than 40 years.

Water from the collection tank gravity-flows into an underground, concrete storage tank (volume about 33 m^3) about 30 m west of the collection tank. A screened overflow pipe returns excess water to the stream. In most years, the storage tank does not overflow during late summer and fall. From the storage tank, water is conveyed through a 10-cm pipe for a horizontal distance of 835 m and a vertical drop of about 12 m and then through 2-cm lateral lines to residential and laboratory buildings.

The water is not chlorinated and, because of the impervious nature of the collection, storage, and conveyance arrangement, is susceptible only to bacterial

contamination by water entering the underground collection pipes. The amount of water used at the headquarters is unknown. However, indicators of water use are that there are usually 10 or more people in residence during the summer; a large residential lawn is maintained by sprinkler irrigation; and a few thousand gallons of water are also hauled to cattle each week from June to October.

Materials and Methods

Bacteria Monitored

Three common indicator bacteria, generally harmless to humans and easily determined quantitatively by routine laboratory procedures, were monitored—total coliforms, fecal coliforms, and fecal streptococci—at irregular time intervals, from March 29 to September 24, 1979. Total coliform bacteria are widespread in fecally contaminated environments (Kabler and Clarke 1960). They originate from diverse sources, including the intestinal tracts of humans, other mammals, birds, reptiles, and lower forms of life. The presence of total coliform bacteria indicates direct or indirect discharge of fecal material into the water. Total coliforms are classified as aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation in 24-48 hours at 35° C (American Public Health Association 1975). This definition includes the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella* (Bordner et al. 1978).

Fecal coliforms, also part of the total coliform group, originate in the intestinal tracts of warmblooded mammals. The major species in the fecal coliform group is *Escherichia coli;* its presence in a stream indicates recent fecal pollution and the possible presence of enteric pathogens. In recently contaminated water, fecal coliforms often represent almost the entire coliform group, but usually the numbers detected are a small portion of the total coliforms. The fecal coliforms are defined as gram-negative rods that ferment lactose in 24 hours at 44.5° C (Bordner et al. 1978).

Fecal streptococci, the third group of microorganisms observed, are a number of species whose presence in water can usually be interpreted as evidence of fecal contamination (Kenner et al. 1960). Two species in the group, *Streptococcus bovis* and *S. equinus* (mainly associated with fecal excrement of nonhuman, warmblooded animals), do not multiply; they survive only a short time in the aquatic environment and can be used as specific indicators of recent contamination. One commonly found species in the group, *S. faecalis*, survives in water longer than *E. coli* (McFeters et al. 1974). This species, and especially one variety (*S. liquefaciens*), is ubiquitous, and its presence can often be attributed to insect activity and other nonanimal sources (Geldreich et al. 1964).

Isolation of Bacteria

Bacteria were recovered from water samples using membrane filtration according to Standard Methods (American Public Health Association 1975). Suitable aliquots of sample were filtered through sterile membranes with a pore diameter of 0.45 mm. Total coliform bacteria retained on the membrane were cultured using commercially prepared m-coliform broth. Plates were checked for growth after incubation for 24 hours at 35° C. Fecal coliforms were recovered after a water bath incubation at 44.5° C for 24 hours on m-FC broth. Fecal streptococci were recovered on KF streptococcal agar and counted after a 48-hour incubation at 35° C. Water samples for bacterial analyses were stored in a refrigerator before filtration. The time period between sampling and filtration was usually less than 1 hour and never more than 6 hours.

On one occasion, tests were also made to characterize fecal streptococci isolates by differential biochemical testing. Colonies were picked from primary isolation plates and were streaked on agar slants of brain heart infusion medium. The slants were incubated for 48 hours, and sample growth was subsequently tested for the presence of catalase. Cultures producing catalase (nonstreptococci) were discarded. Catalase-negative organisms were further classified according to procedures outlined by Bordner et al. (1978).

Sample Sites

Six sampling sites were selected for monitoring bacterial populations (fig. 1). The approximate locations of the six sites were as follows:

Site	Stream distance above collection tank
1	1.6 km (50 m upstream from first home)
2	1.4 km (50 m downstream from first and second home)
3	1.0 km (50 m downstream from fourth home)
4	0.8 km (50 m downstream from fifth home)
5	Collection tank
6	Water tap in station laboratory

Shallow groundwater wells (0.7-1.5 m deep), cased with clean, 5-cm-diameter, polyethylene pipe, perforated to within 60 cm of the ground surface, were also established adjacent to the stream at sites 1, 2, 3, and 4 to assure collection of water samples for as long as possible. Surface flow was continuous at sites 1 and 2 during the study. Water samples at site 5 were taken from the underground collection tank. Water samples at site 6 were taken from a water tap in the headquarters laboratory after running it for 5 minutes before collection.

Alluvium Texture

The stream alluvium was sampled and sieved (air dry) on one occasion to determine particle sizes adjacent to the perforated pipe intake of the water collection tank.

Soil samples were collected in 30-cm depth increments to 120 cm, below which groundwater seepage prevented further soil collection. Stream alluvium near the other sample sites was similar to that observed at the collection tank.

Results and Discussion

Total Coliforms

The interim, primary standard set forth for the Safe Drinking Water Act (U.S. Public Law 93-523) proposed that the total coliform bacteria group average less than 1 colony/100 ml for potable water (U.S. Environmental Protection Agency 1975). Total coliforms observed at sites 1, 2, 3, and 4 (both streamwater and streambed wells), from June through September, were well above the recommended safe level for potable water (table 1). The high recovery of total coliforms from water samples taken at sites 2 and 3 did not appear to result from the nearby home areas, because high counts were also observed at site 1 located about 50 m upstream from the uppermost home area. Throughout the monitoring period, counts of total coliforms were much higher at the upper sample sites compared to water samples taken at sites 5 and 6 (water collection tank and tap, respectively). Total coliforms were highest during the periods July 10-13 and August 21-24 at most upstream sites, but at the same time, they remained relatively low at the point of diversion and in the domestic water supply. The high upstream counts appeared to indicate a

Table 1.—Mean counts of total coliforms, fecal coliforms, and fecal streptococci in water samples for selected periods

Indicator bacteria and date	Observations	Sampling sites[1]									
		1s	1w	2s	2w	3s	3w	4s	4w	5	6
		colonies/100 ml									
Total coliforms											
Mar. 29	1	0	—	0	—	0	—	0	—	0	0
Apr. 28	1	8	—	11	—	4	—	4	—	1	0
May 24	1	6	—	0	—	2	—	4	—	0	0
June 7- 8	2	1	—	2	—	2	—	2	—	0	0
12-14	4	4	45	4	—	2	—	3	< 1	1	0
20-21	3	11	—	5	—	11	—	44	—	< 1	0
26-27	3	16	—	15	—	12	—	—	—	0	0
July 10-13	4	130	950	315	341	—	36	—	28	2	2
24-27	7	6	4	36	6	—	1	—	0	0	0
Aug. 6-10	7	54	2	54	2	—	0	—	0	0	0
21-24	5	1200	2200	620	850	—	1000	—	38	21	4
Sept. 4- 7	4	120	12	18	3	—	0	—	0	0	0
21-24	4	320	72	230	100	—	0	—	10	6	2
Fecal coliforms											
Apr. 28	1	0	—	0	—	1	—	0	—	0	0
June 12-14	4	< 1	0	0	—	0	—	0	—	0	0
July 10-13	4	0	0	11	< 1	—	0	—	0	0	0
24-27	7	< 1	0	1	0	—	0	—	0	0	0
Aug. 6-10	7	0	0	2	0	—	0	—	0	0	0
21-24	5	0	0	< 1	0	—	0	—	0	0	0
Sept. 4- 7	4	2	0	11	0	—	0	—	0	0	0
21-24	4	0	0	1	0	—	0	—	0	0	0
Fecal streptococci											
Mar. 29	1	150	—	620	—	470	—	580	—	1	63
Apr. 28	1	110	—	10	—	120	—	90	—	10	150
May 24	1	10	—	30	—	50	—	290	—	50	10
June 7- 8	2	78	—	52	—	55	—	360	—	10	10
12-14	4	46	48	64	—	38	—	690	0	24	6
20-21	3	91	—	88	—	66	—	1900	—	18	8
26-27	3	75	—	74	—	109	—	—	—	21	0
July 10-13	4	1000	2100	720	970	—	9000	—	530	5	2
24-27	7	1300	790	1200	220	—	820	—	94	32	6
Aug. 6-10	7	3100	6400	2700	230	—	770	—	60	38	37
21-24	5	2900	7600	2300	590	—	1700	—	180	25	7
Sept. 4- 7	4	1000	700	1400	300	—	460	—	120	31	5
21-24	4	220	69	90	46	—	TNTC[2]	—	0	2	1

[1]*s = stream sample; w = groundwater sample*
[2]*Too numerous to count*

sporadic input of bacterial contamination that was substantially diluted and/or partially filtered before reaching the potable water collection and distribution system.

Fecal Coliforms

Fecal coliform organisms were not detected at sample sites 4, 5, and 6 and only infrequently detected at the upper three sites (table 1). The greatest number of colonies detected at site 1, above the home areas, was five on September 7. On two occasions (July 12 and September 7), 44 colonies/100 ml of water were found at site 2. This site is 50 m below the uppermost home, which has its septic tank near the stream channel. Only on one occasion (July 13) was a fecal coliform colony observed in the groundwater well at site 2. Fecal coliforms were not found in any other groundwater well. The low fecal coliform counts apparently indicated Hotel Gulch was receiving little direct input of warmblooded animal feces.

Fecal Streptococci

Fecal streptococci were found regularly in each of the six sampling sites throughout the study period (table 1). There tended to be relatively more fecal streptococci at sites 1, 2, 3, and 4 and relatively fewer in the domestic water collection tank and water tap. Fecal streptococci isolates from Hotel Gulch were found to consist mostly of *S. faecalis* (63%), with *S. avium* and *S. equinus* each accounting for 15% of the total; the remaining 7% were characterized as *S. bovis* (2%) and as unknown (5%).

Precipitation and Bacteria Counts

Mean bacteria counts reached their peak in August (fig. 2). The high counts in August were not significantly correlated with rainfall. The rain showers that occurred were also not large enough to greatly change the flow regime. Streamflow and water temperature (early morning readings) in Hotel Gulch, measured periodically at an established station 2 km above site 1, fluctuated only slightly in response to summer rains:

Date observed		Flow	Temperature
		l/s	°C
July	10	4.8	7.5
	21	4.8	10.0
Aug.	7	2.3	12.0
	21	4.5	6.0
	28	4.0	8.0
Sept.	11	3.1	7.5

No definitive increased human or animal activities were observed during July and August. High counts of bacteria in July and August were perhaps a cumulative result of numerous, small, high-intensity afternoon showers, averaging generally less than 10 mm. Studies in other mountainous areas in Colorado have generally shown that large rainshowers and melting snow significantly increase bacteria concentrations by flushing bacteria directly into streams (Morrison and Fair 1966, Kunkle and Meiman 1967).

Alluvium Texture and Bacteria Counts

Surface flow ceased at site 4 (0.8 km above the collection tank) about June 24. After this date and until the study was terminated, there was no flowing or standing water in the stream channel between site 4 and the collection tank. All water entering the collection tank was thus derived from groundwater flow. Figure 3 illustrates percent weight distribution of selected sizes of particles making up the stream channel alluvium adjacent to the collection tank. Aquifer materials best suited for filtering biological contaminants are those reported to be uniformly composed of very fine (0.063-0.125 mm) to fine (0.125-0.250 mm) textured sand with high clay content (Romero 1970, Boyd et al. 1969). Based on the relatively coarse-textured and widely variable alluvium, there was apparently incomplete bacterial filtering of groundwater flowing into the collection tank.

Streamflow and its associated bacterial concentrations above site 4 are not simply related to groundwater flow in the collection tank; data in figure 2 provides further evidence of partial but incomplete bacterial filtering. During late July, August, and early September, relatively high bacterial counts in the water collection tank were generally proportional to high bacterial counts in the streamflow. During August, counts of fecal streptococci in flowing surface water at the two upper study sites were about 75 times greater and total coliform counts about 45 times greater than bacterial concentrations in water contained in the collection tank.

Conclusions and Recommendations

Hotel Gulch stream water was found to contain coliform and fecal streptococcal bacteria consistently. Fecal coliforms were found only sporadically and in very low numbers. The stream contamination with indicator bacteria seems to be associated with fecal materials in the soil and vegetation in the watershed rather than direct fecal inputs from man or other warmblooded animals. The levels of bacteria were not especially great for a small mountain stream and showed no increase in those samples taken near the upstream homes during the spring, summer, and fall months of 1979; this indicated the seldom-occupied cabins and the permanent home did not discharge detectable quantities of fecal material into the stream. The precipitation pattern during late July and August appeared to cause temporary increases in the number of bacteria, as was reflected by the coliform bacteria found in the drinking water used in the forest compound. The coarse-textured alluvium near the collection tank also provided evidence of partial but incomplete bacterial filtering.

The periodic presence of coliform bacteria indicated that the untreated domestic water supply of the Manitou

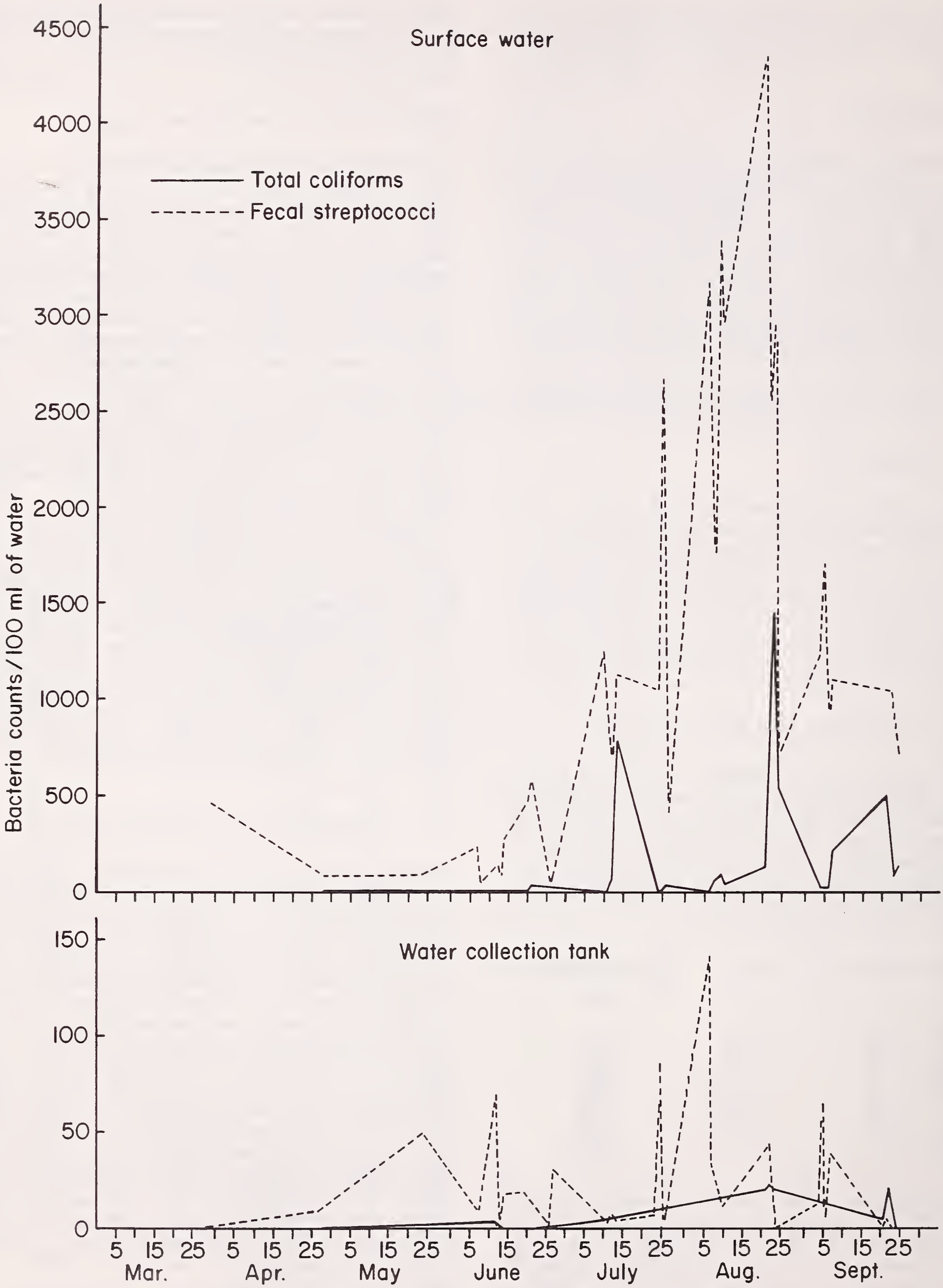

Figure 2.—Mean counts of total coliforms and fecal streptococci in flowing surface water and in the water collection tank.

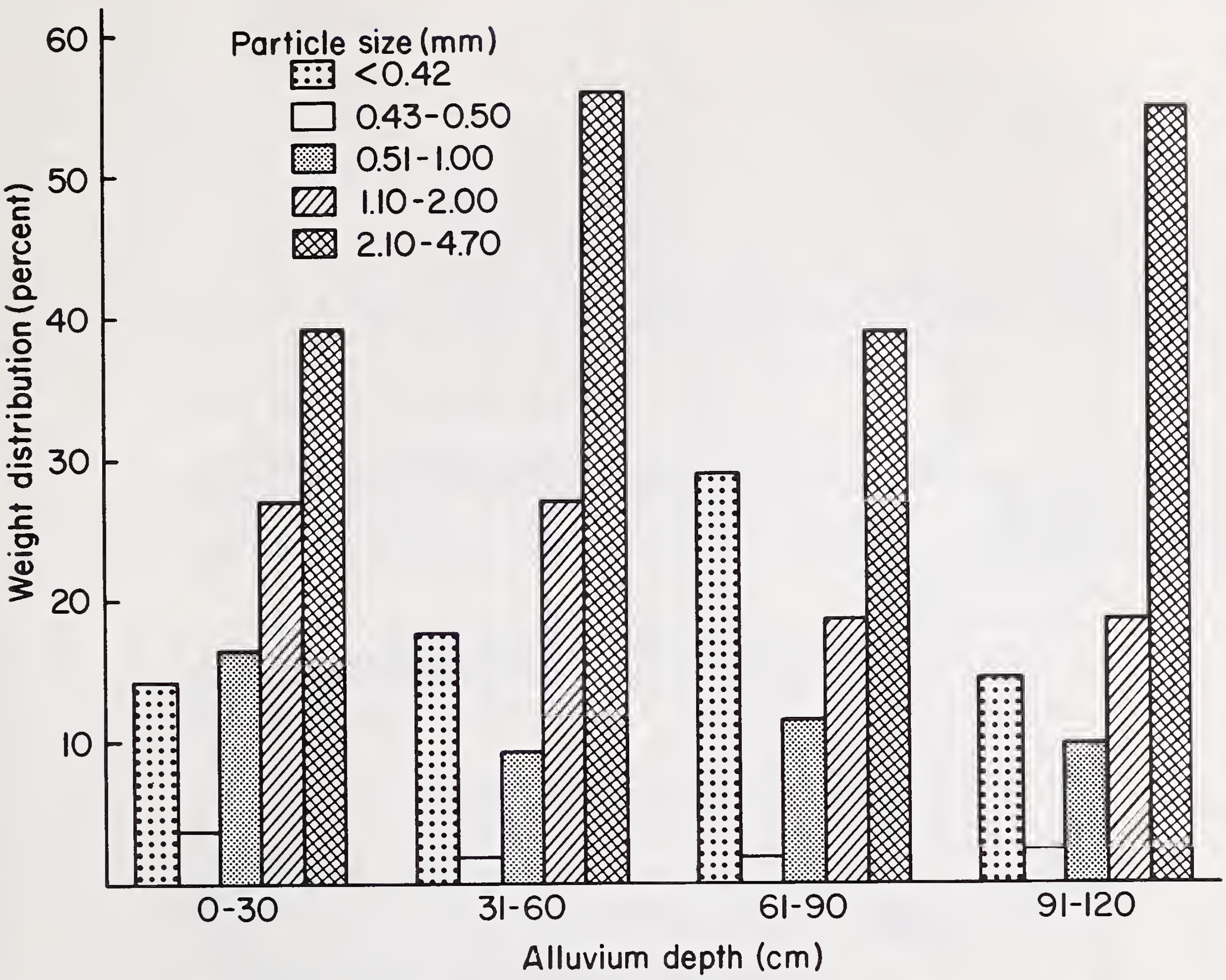

Figure 3.—Percent weight distribution of selected particle sizes of alluvium adjacent to the collection tank.

Experimental Forest is incompletely filtered. Therefore, it is necessary that remedial action be taken to eliminate the potential health hazard. Two possible courses of action are chlorination and/or establishment of a new well that is pumped from a deeper aquifer, or preferably both.

Literature Cited

American Public Health Association. 1975. Standard methods for the examination of water and wastewater. 14th edtion. 1193 p. American Public Health Association, Inc., New York, N.Y.

Boyd, J. W., T. Yoshida, L. E. Vereen, R. L. Cada, and S. M. Morrison. 1969. Bacterial response to the soil environment. Colorado State University Sanitary Engineering Paper Number 5, p. 10-15. Fort Collins.

Bordner, Robert H., John A. Winter, and Pasquale Scarpino. 1978. Microbiological methods for monitoring the environment. EPA-600/8-78-017, 338 p. United States Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio.

Colorado Water Quality Control Commission. 1978. Water quality standards for Colorado. 27 p. plus 17 p. appendix. Colorado Department of Health, Denver.

Geldreich, E. E., B. A. Kenner, and P. W. Kabler. 1964. Occurrence of coliforms, fecal coliforms and streptococci on vegetation and insects. Applied Microbiology 12(1):63-69.

Kabler, P. W., and H. F. Clarke. 1960. Coliform group and fecal coliform group organisms as indicators of pollution in drinking waters. Journal of American Water Works Association 52:1577-79.

Kenner, B. A., H. F. Clarke, and P. W. Kabler. 1960. Fecal streptococci. II. Quantification of streptococci in feces. American Journal of Public Health 50:1553-1559.

Kunkle, Samuel H., and James R. Meiman. 1967. Water quality of mountain watersheds. Colorado State University Hydrology Paper Number 21, 53 p. Fort Collins.

McFeters, Gordon A., Gary K. Bissonette, J. H. Hezeski, C. A. Thomson, and David G. Stuart. 1974. Comparative survival of indicator bacteria and enteropathogens in well water. Applied Microbiology 24:823-829.

Morrison, S. M., and J. F. Fair. 1966. Influence of environment on stream microbial dynamics. Colorado State University Hydrology Paper Number 13, p. 14-15. Fort Collins.

Romero, John C. 1970. The movement of bacteria and virus through porous media. Ground Water 8:37-48.

U.S. Environmental Protection Agency. 1975. Interim primary drinking water standards. Federal Register 40(51):11, 990-11998.

Agriculture—CSU, Fort Collins

Rocky
Mountains

Southwest

Great
Plains

U.S. Department of Agriculture
Forest Service

Rocky Mountain Forest and Range Experiment Station

The Rocky Mountain Station is one of eight regional experiment stations, plus the Forest Products Laboratory and the Washington Office Staff, that make up the Forest Service research organization.

RESEARCH FOCUS

Research programs at the Rocky Mountain Station are coordinated with area universities and with other institutions. Many studies are conducted on a cooperative basis to accelerate solutions to problems involving range, water, wildlife and fish habitat, human and community development, timber, recreation, protection, and multiresource evaluation.

RESEARCH LOCATIONS

Research Work Units of the Rocky Mountain Station are operated in cooperation with universities in the following cities:

Albuquerque, New Mexico
Bottineau, North Dakota
Flagstaff, Arizona
Fort Collins, Colorado*
Laramie, Wyoming
Lincoln, Nebraska
Lubbock, Texas
Rapid City, South Dakota
Tempe, Arizona

*Station Headquarters: 240 W. Prospect St., Fort Collins, CO 80526